In re application of: Lonnie O. Ingram, *et al.*
Application No.: 09/885,294
Group No.: 1651
Filed: June 19, 2001

REMARKS

Claims 1, 3, 4, 6-11, 18, 19, 21-33 and 36-42 were pending in the application. Claim 1 has been amended and claim 8 has been cancelled without prejudice or disclaimer. Accordingly, claims 1, 3, 4, 6, 7, 9-11, 18, 19, 21-33 and 36-42 will be pending in the application upon entry of this amendment.

Support for the amendment of claim 1 can be found throughout the specification and claims as originally filed. In particular, support can be found in the specification at least, for example, at page 10, lines 34-37, in Examples 1 and 2, and in claims 1 and 8 as originally filed .

Amendment of the claims is not to be construed as an acquiescence to any of the rejections/objections set forth in the instant Office Action or in any previous Office Action, and were done solely to expedite prosecution of the application. Applicants reserve the right to pursue the claims as originally filed, or substantially similar claims, in this or one or more continuation patent applications.

Applicants invite the Examiner's attention to the current amendment to claim 1, which restores the definition of the R_1 substituent in formula I to the definition originally filed. Should the Examiner reinstate the obviousness-type double patenting rejection over U.S. Patent 6,130,076 that was made in the previous office action, Applicants will consider filing a terminal disclaimer to overcome the rejection upon a finding that the application is otherwise in condition for allowance.

Claims Rejections – 35 U.S.C. §112, Second Paragraph

Claims 1 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner alleges that the nature of the term "control" in claim 1 is not specified. Applicants respectfully disagree and traverse the rejection.

However, without acquiescing to the rejection and in order to expedite prosecution of the application, claim 1 has been amended in accordance with the Examiner's helpful suggestion on page 2 of the Office Action. Specifically, claim 1 now recites "as compared to an alcohologenic

recombinant control cell that is cultured under the same conditions in the absence of the compound of formula I".

Claims Rejections – 35 U.S.C. §102

Claims 1, 3, 4, 10, 11, 18, 19, 21, 22, 23, 25, 27, 30, 31, 32, 38, 39 and 40 are rejected under 35 U.S.C. §102(e) as being anticipated by Stanley, G.A., *et al.*, "Inhibition and Stimulation of Growth by Acetaldehyde," *Biotechnology Letters* **15(12)**: 1199-1204 (December 1993) ("Stanley, *et al.* 1993"). Applicants disagree and respectfully traverse the rejection.

However, without acquiescing to the rejection and in order to expedite prosecution of the application, claim 1 has been amended by incorporating the recitation of claim 8, a claim that was not subject to the rejection. Accordingly, Applicants submit that the rejection no longer applies to claim 1, and the claims depending therefrom, and respectfully request reconsideration and withdrawal of the rejection.

Claims 1, 3, 4, 10, 11, 18, 19, 21, 22, 23, 25, 27, 30, 31, 32, 38, 39 and 40 are rejected under 35 U.S.C. §102(b) as being anticipated by Stanley, G.A., *et al.*, "Effect of Acetaldehyde on *Saccharomyces cerevisiae* and *Zymomonas mobilis* Subjected to Environmental Shocks" *Biotechnology and Bioengineering* **53**: 71-78 (1997) ("Stanley, *et al.* 1997)". Applicants disagree and respectfully traverse the rejection.

However, without acquiescing to the rejection and in order to expedite prosecution of the application, claim 1 has been amended by incorporating the recitation of claim 8, a claim that was not subject to the rejection. Accordingly, Applicants submit that the rejection no longer applies to claim 1, and the claims depending therefrom, and respectfully request reconsideration and withdrawal of the rejection.

Claims Rejections – 35 U.S.C. §103

Claims 1, 3, 4, 6-11, 18, 19, 21-33 and 36-42 are rejected under 35 U.S.C. §103(a) as being unpatentable over Stanley, *et al.* 1993 or Stanley, *et al.* 1997, taken with U.S. Patent 6,107,093 to Ingram ("Ingram"). Applicants disagree and respectfully traverse the rejection.

Specifically, the Examiner alleges that both Stanley, *et al.* references disclose a process for producing ethanol with a cell by contacting a medium containing glucose with ethanologenic cells of *Saccharomyces cerevisiae* and contacting the cells with acetaldehyde. The Examiner also alleges that the production of alcohol is increased, because there is reduced inhibition due to ethanol as evidenced by an increase in growth of the cells. The Examiner admits that the Stanley, *et al.* references neither teach nor suggest the use of recombinant ethanologenic cells such as the *E. coli* and *Klebsiella* strains recited in claim 9 of the invention. However, the Examiner asserts that Ingram demonstrates that recombinant ethanologenic cells such as the *E. coli* and *Klebsiella* strains are old and well known. Based on the disclosure in Ingram at column 3, lines 42 *et seq.*, the Examiner concludes that it would have been obvious to one of ordinary skill in the art to modify the process of either Stanley, *et al.* reference by exposing alcohologenic cells to intermediates in the metabolic pathways of alcohol production, other than acetaldehyde, particularly in alcohologenic cells in which the metabolic pathways for alcohol production have been altered. Applicants respectfully disagree.

Claim 1 has been amended to include the recitation of claim 8. Thus, claim 1, as presented herein, is directed to a method for increasing production of alcohol, which method uses a ***recombinant*** alcohologenic cell. Applicants respectfully submit that none of the cited references, alone or in combination, teach or suggest the invention recited in claim 1 as presented herein, and the claims depending therefrom.

The Stanley, *et al.* 1993 references disclose that low concentrations of acetaldehyde reduced the lag phase in ethanol-containing medium and increased the specific growth rate of yeast, specifically *Saccharomyces cerevisiae*. As admitted by the Examiner, there is no teaching or suggestion in either of the references to use recombinant ethanologenic cells, *e.g.*, recombinant members of the family Enterobacteriaceae, of which family *E. coli* and *Klebsiella* are members and are not naturally ethanologenic. In other words, neither reference teaches or suggests using a cell which, unlike *Saccharomyces cerevisiae*, is not naturally ethanologenic, but which has been engineered to express heterologous genes, the expression of which render the recombinant cells alcohologenic, *e.g.*, ethanologenic.

Ingram discloses and claims recombinant cells that highly express chromosomally integrated heterologous genes, *e.g.*, *Zymomonas mobilis* pyruvate decarboxylase (*pdc*) and alcohol dehyrogenase (*adh*), such that the recombinant cells, unlike their parent strains such as the *E. coli* and *Klebsiella* strains that are not naturally ethanologenic, become ethanologenic. In other words, the metabolic pathways of the *E. coli* and *Klebsiella* strains, which ordinarily produce carboxylic acids as the major metabolic byproducts, have been altered by genetic engineering such that the major metabolic byproduct is ethanol. Ingram is completely silent as to exposing the recombinant ethanologenic cells disclosed therein to acetaldehyde or other metabolic intermediates to increase ethanol production.

There is nothing in any of the cited references that would motivate one of ordinary skill in the art to combine the references in the manner suggested by the Examiner. One of ordinary skill in the art will readily appreciate that *Saccharomyces cerevisiae*, the subject of the Stanley, *et al.* references, is a species of yeast that is naturally ethanologenic. Unlike Ingram, there is no teaching or suggestion in either of the Stanley, *et al.* references to alter the metabolic pathway of *Saccharomyces cerevisiae* by genetic engineering. Accordingly, one of ordinary skill in the art would have no reason to genetically engineer *Saccharomyces cerevisiae*, in the manner taught by Ingram, to make the yeast species ethanologenic, because the yeast species is already naturally ethanologenic. Thus, there is nothing in the Stanley, *et al.* references that would prompt one of ordinary skill in the art to genetically engineer *Saccharomyces cerevisiae* as taught in Ingram.

Indeed, ethanol production was not a problem in the Stanley, *et al.* references. The problem encountered in these references was increase in lag phase and decrease in cell growth because of an increase in ethanol concentration in the culture medium. This problem was addressed by adding acetaldehyde to the culture medium whereby, at certain concentrations of acetaldehyde, lag phase was decreased and cell growth was increased.

In contrast, the problem solved by Ingram was how to make organisms that are not naturally ethanologenic become ethanologenic. Ingram solved this entirely different problem by engineering the organisms to express heterologous ethanol producing genes (*Z. mobilis* alcohol dehydrogenase and pyruvate decarboxylase genes) from a different organism.

The Examiner cites column 3, lines 42 *et seq.* of Ingram to support the position that one of ordinary skill in the art would have a reasonable expectation of success in improving ethanol production by exposing alcohologenic cells to other intermediates, *e.g.*, pyruvic acid, succinic acid, *etc.*, in the metabolic pathway leading to ethanol production. However, this citation does not support such a position.

Column 3, lines 42 *et seq.* of Ingram is addressed solely to expression of *Z. mobilis* alcohol dehydrogenase and pyruvate decarboxylase genes in certain enteric bacteria in order to make such organisms ethanologenic. The passage goes on to teach that ethanol production can be made more efficient by transforming the enteric organisms with multi-copy plasmids bearing these heterologous genes. There is no mention whatsoever of exposing the recombinant cells to any other intermediates in the ethanol metabolic pathway to increase ethanol production.

In fact, pyruvate decarboxylase is an enzyme that breaks down pyruvic acid by removing the carboxylic acid moiety from a pyruvic acid molecule. With this knowledge, one of ordinary skill in the art would construe column 3, lines 42 *et seq.* of Ingram as teaching away from adding pyruvic acid, acetaldehyde or other compounds encompassed by formula I of claim 1.

Moreover, even if Ingram did suggest exposing alcohologenic cells to metabolic pathway intermediates such as acetaldehyde, there is nothing in Ingram or the Stanley, *et al.* references to suggest that bacteria such as *E. coli*, *Klebsiella* and *Erwinia* would behave the same way as the *Saccharomyces cerevisiae* of the Stanley, *et al.* references upon exposure to acetaldehyde. The former are gram-negative enteric bacteria whereas the latter are gram positive, spore-forming yeast. There is nothing in any of the references to lead one of ordinary skill in the art to expect that recombinant enteric bacteria would survive upon exposure to acetaldehyde, much less increase ethanol production. Even Stanley, *et al.* 1993 indicates that at certain concentrations, acetaldehyde was toxic to *Saccharomyces cerevisiae*. It would be anyone's guess as to what would be the outcome of exposing the recombinant enteric bacteria of Ingram to acetaldehyde in the manner taught by Stanley, *et al.*

"Obvious to try" is not the test of obviousness. Rather, the cited references must teach or suggest that one of ordinary skill in the art ***should*** modify the teachings of the references to arrive at the claimed invention. The situation here is at best a case of obvious to try.

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Applicants submit that the Examiner has failed to establish a *prima facie* showing of obviousness. In fact, the rejection as presented in the Office Action is nothing more than an impermissible hindsight reconstruction of the invention based on Applicants' own teachings. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

CONCLUSION

In view of the foregoing, reconsideration and withdrawal of all rejections and allowance of the application with claims 1, 3, 4, 6, 7, 9-11, 18, 19, 21-33 and 36-42 are respectfully solicited. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Respectfully submitted,
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